

Molecular Phylogenetic Analysis of the Genus *Symplocarpus* (Araceae) from Japan Based on Chloroplast DNA Sequences

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Phylogenetic relationships among three species of the genus *Symplocarpus* were examined based on nucleotide sequences of the chloroplast DNA *trnG* intron (847bp) and 3' to *rps2* (935bp) regions. Of these regions of cpDNA, a total of 11 (0.6 %) nucleotide positions were variable and three species-specific haplotypes were identified among five samples of *Symplocarpus*. The phylogenetic analysis indicates *S. nabekuraensis* is closer to *S. foetidus* rather than to *S. nipponicus*.

Key words: 3' to *rps2*, chloroplast DNA, molecular phylogeny, sequences, *Symplocarpus*, *trnG* intron.

Species of the genus *Symplocarpus* (Araceae) are hermaphroditic perennial herbs blooming from spring to early summer under humid, broad-leaved deciduous forests. The genus consists of three species from Eastern Asia and North America (Ohwi 1978, Ohashi 1982, Otsuka et al. 2002). *Symplocarpus nabekuraensis* Otsuka & K. Inoue is a recently recognized species from northern Nagano Prefecture of central Japan, which differs from *Symplocarpus foetidus* Nutt. var. *latissimus* (Makino) H. Hara (syn. *S. renifolius* Schott ex Miq.) and *Symplocarpus nipponicus* Makino in morphological and ecological traits (Otsuka et al. 2002). The distribution of *S. nabekuraensis* is restricted to snowy regions in the Hokuriku and Tohoku districts of Honshu, whereas those of the other two species extended to Honshu and Hokkaido Islands in Japan (Otsuka 2002). The variation ranges of leaf length and spathe size of *S. nabekuraensis* overlap with those of *S. nipponicus*, but the

reniform leaf blade and major phenological traits are similar to *S. foetidus* (Otsuka et al. 2002). Phenologically fruiting of *S. nipponicus* occurs during the season following blooming, while that of the other species occurs during the same season as blooming (Otsuka et al. 2002). According to these general similarities, *S. nabekuraensis* is believed to have a closer phylogenetic relationship to *S. foetidus* rather than with *S. nipponicus*. Previous molecular phylogenetic analyses for the genus *Symplocarpus* (Wen et al. 1996) did not include *S. nabekuraensis*.

The aim of this study is to examine genetic relationship among three species of genus *Symplocarpus*. We used DNA sequences of intergenic spacers of the chloroplast DNA (cpDNA), which are relatively fast evolving and widely used in systematic studies at lower taxonomic ranks (e. g., Kelchner and Clark 1997, Weising and Gardner 1999, Friesen et al. 2000).

Materials and Methods

Plant materials

We used one sample of *S. nabekuraensis* from the type locality (Iiyama City) and two samples each of *S. nipponicus* and *S. foetidus*, respectively (Fig. 1). For *S. nipponicus* and *S. foetidus*, which are known for their wide morphological diversity, we sampled two varieties (morphological types) to assess the intraspecific variation of genetic markers [N = 2 for *S. nipponicus* including different color forms (Ohara 1985) and N = 2 for *S. foetidus* including two ecotypes; Fig. 1]. An ecotype of *S. foetidus* inhabiting snowy mountain areas has phenological characteristics similar to *S. nabekuraensis* as the leaves emerge simultaneously with spathe emergence (Otsuka 2002). We used *Lysichiton camtschatcense* (L.) Schott as an outgroup species, which is sister to *Symplocarpus* (French et al. 1995). The source of plant materials and vouchers of the accessions used in this study are listed in Table 1.

DNA isolation and PCR

Total genomic DNA was extracted from leaves of each species with a DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. Concentrations of DNA samples were measured with a spectrophotometer (Shimazu UV-1200) to unify the experimental conditions.

To confirm genetic variation among samples, we conducted sequence analysis of two regions: *trnG* intron and 3' to *rps2* (Tsumura et al. 2000). The primer sequences were 5'-GCG GGT ATA GTT TAG TGG TA-3' (forward) & 5'-CCT CTG TCC TAT CCA TTA GAC-3' (reverse) for *trnG* intron, and 5'-GTC ATA TAT TTG ATC CCG CC-3' (forward) & 5'-CGA GTT TTA GCA AAA GCT GC-3' (reverse) for 3' to *rps2*. PCR was carried out in a Perkin Elmer thermal cycler programmed for 95 °C–11 min [94 °C–30 sec, 45 °C–30 sec, 72 °C–2 min] × 30 cycles and 72 °C–7min. Amplifications were

carried out in 50-μL reaction volumes including 1 U Taq DNA polymerase (ABI AmpliTaq Gold), 1X PCR buffer (ABI), 0.2 mM each dNTP (ABI), 8 pmol each primer, and 5 ng of total DNA. The PCR products were electrophoresed on a 6 % agarose gel, cut out, and purified with the NucleoSpin Extract kit (Macherey-Nagel). After checking DNA concentration on a 1.8 % agarose gel, about 40 ng of the PCR product was used in a 10 μL-cycle sequencing reaction with the ABI BigDye Terminator Kit (ver. 3) on an ABI 377 DNA sequencer (ABI, Foster City, CA). Forward and reverse sequences were determined for each taxon and combined into a single consensus sequence.

Data analysis

Phylogenetic analyses using the maximum parsimony method were performed with PHYLIP 3.57c program package (Felsenstein 1995). DNA sequences of *trnG* intron and 3' to *rps2* were combined initially, and multiple alignments were performed using ClustalW (Thompson et al. 1994). The genetic relationships among mtDNA haplotypes were assessed by the F84 model (Kishino and Hasegawa 1989) after the data were bootstrapped (1000 times) using the program SEQBOOT and DNAPARS (Felsenstein 1985). A consensus dendrogram was then constructed to estimate the confidence level of the obtained tree by the CONSENSE program. The pairwise sequence divergence between haplotypes was estimated as percent nucleotide substitutions per site.

Results and Discussion

The sequenced lengths of cpDNA region in *Symplocarpus* were 846–847bp for the *trnG* intron: and 930–932 bp for 3' to *rps2* (Table 2). When these two regions of cpDNA were combined, a total of 11 (0.6 %) nucleotide positions were variable and three haplotypes were identified. Intraspeci-

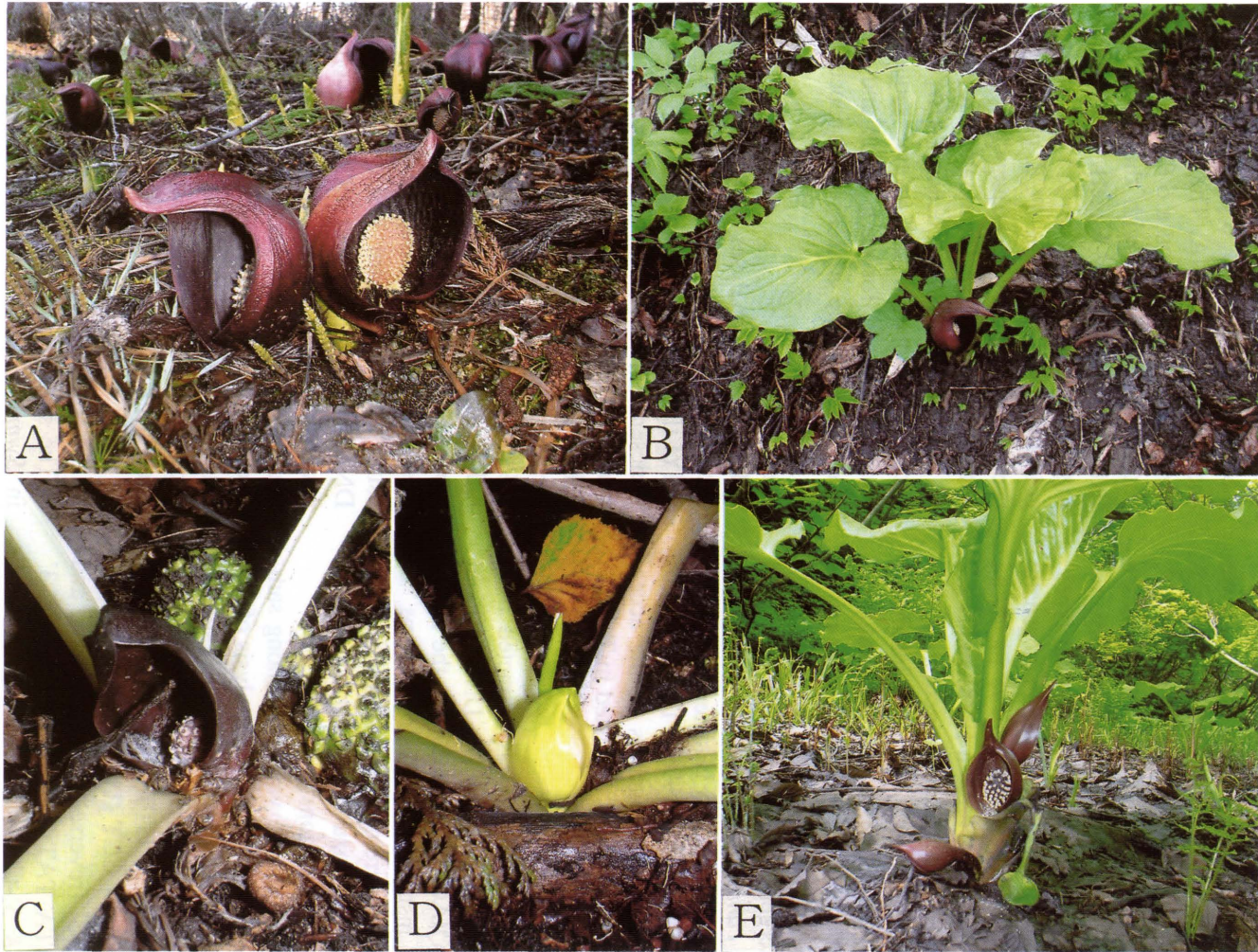


Fig. 1. *Symplocarpus* species examined in this study. A: *S. foetidus* var. *latissimus* (Hakuba Village; 28 April, 2003). B: An ecotype of *S. foetidus* var. *latissimus* adapted to heavily snowy climate (Arai City; 26 June, 2003). C: *S. nipponicus* (Nagano; 23 June, 2002). D: *S. nipponicus* f. *viridispatius* (Ohoka, Nagano; 29 June, 2003). E: *S. nabekuraensis* (Iiyama City; 28 June, 2002).

Table 1. Source of *Symplocarpus* materials for molecular analysis and DDBJ accession number of the cpDNA (*trnG* intron and 3' to *rps2*)

Taxon	Locality	Haplotype	DDBJ Accession number	
			<i>trnG</i> intron	3' to <i>rps2</i>
<i>S. foetidus</i> var. <i>latissimus</i>	Hakuba, Nagano Pref.	Hap-1	AB206656	AB206660
	Arai, Niigata Pref.	Hap-1		
<i>S. nabekuraensis</i>	Iiyama (Mt. Nabekura), Nagano Pref.	Hap-2	AB206658	AB206662
<i>S. nipponicus</i>	Iizuna, Nagano Pref.	Hap-3	AB206657	AB206661
<i>S. nipponicus</i> f. <i>viridispathus</i>	Ohoka, Nagano Pref.	Hap-3		
<i>Lysichiton camtschatcense</i>	Iizuna, Nagano Pref.		AB206655	AB206659

Table 2. Chloroplast DNA sequence variation from the *trnG* intron (847bp) and 3' to *rps2* (935bp) regions of *Symplocarpus* and *Lysichiton camtschatcense*. Only variable nucleotide positions are shown

Haplotype	<i>trnG</i> intron (847bp)									3' to <i>rps2</i> (935bp)															
	1	1	2	2	2	3	5	6	7		4	4	4	4	4	4	4	6	6	6	6	8			
	5	6	2	4	4	8	2	1	6	1	5	5	6	2	7	8	8	8	9	9	4	4	5	5	5
	2	7	0	4	5	5	1	0	6	4	0	1	2	7	9	0	1	2	4	5	8	9	0	5	3
Hap-1	A	C	G	T	A	A	C	T	G	A	G	G	G	C	T	T	A	A	—	—	—	—	—	T	G
Hap-2	.	.	.	G	.	C	A	.	A	.	.	.	.	.	.	.	.	A	A	—	—	—	.	.	
Hap-3	.	T	A	.	G	C	.	C	.	G	.	.	.	T	—	—	.	A	—	—	—	—	.	T	
<i>Lysichiton camtschatcense</i>	.	.	.	.	.	.	.	.	.	.	T	T	T	T	.	.	C	T	A	—	A	A	A	A	T

fic sequence variation was not observed for the different ecotypes of *S. foetidus* or color forms of *S. nipponicus*. The pairwise sequence divergences among three species of *Symplocarpus* were 0.22 % (*S. nabekuraensis* vs. *S. foetidus*), 0.45 % (*S. nipponicus* vs. *S. foetidus*) and 0.56 % (*S. nabekuraensis* vs. *S. nipponicus*). *Symplocarpus nabekuraensis* is closer to *S. foetidus* than to *S. nipponicus*, as is indicated by the cluster in the most parsimonious tree (84 % bootstrap support, Fig. 2).

The results of the molecular analyses are congruent with general similarities as observed in leaf morphology, leaf phenology and fruiting season (Otsuka et al. 2002). It is plausible that genetic criterion exist among three species of *Symplocarpus* since no difference in cpDNA sequences between two

distinctive morphological types from *S. foetidus* or *S. nipponicus* was evident. Molecular analyses by Wen et al. (1996), namely those focusing on cpDNA restriction site data, indicated a significant genetic difference between specimens of *S. foetidus* from Japan (originally described as *S. renifolius*) and North America. In order to confirm the validity of the three species further analyses of additional specimens across the geographically extensive populations should be provided.

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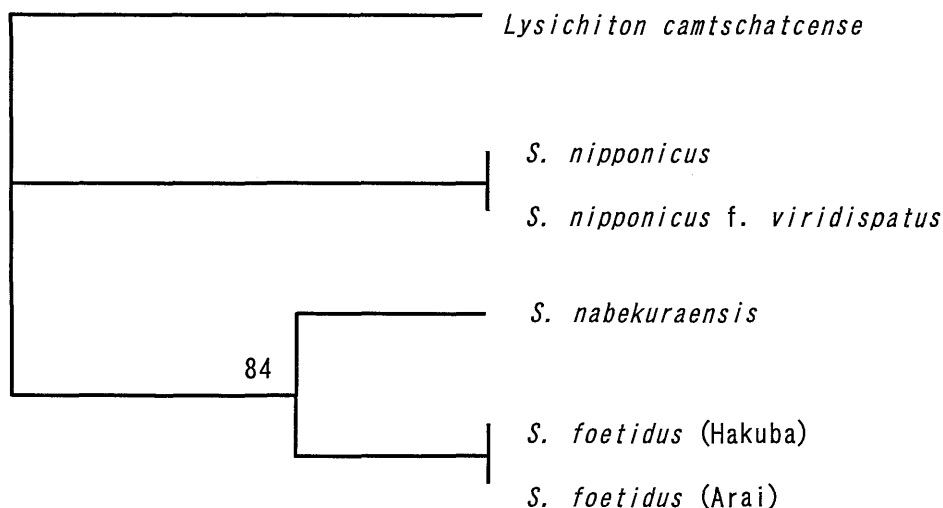


Fig. 2. The single most parsimonious tree for the genus *Symplocarpus* based on the combined cpDNA sequences of *trnG* intron and 3' to *rps2*. Percentage of 1,000 bootstrap replications is given above branches.

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北野 聡^a, 大塚孝一^a, 上杉龍士^b, 五箇公一^c: 葉
緑体 DNA の塩基配列変異に基づく日本産ザゼン
ソウ属 3 種の系統関係

新種として記載されたナベクラザゼンソウ
Symplocarpus nabekuraensis の系統的な位置を明ら
かにする目的で、北信越地方から得られたザゼン
ソウ *S. foetidus* var. *latissimus*, ヒメザゼンソウ *S.*
nipponicus, ナベクラザゼンソウ, ミズバショウ
Lysichiton camtschatcense の計 6 標本を対象に、葉
緑体 DNA の *trnG* intron (847 bp) ならびに 3' to
rps2 (935 bp) 領域の塩基配列を解析した。その
結果、ナベクラザゼンソウはヒメザゼンソウより

もザゼンソウとの遺伝的類似性が高く、近縁であ
ることがわかった。また、ヒメザゼンソウ、ザゼ
ンソウについては形態の異なる品種（あるいは生
態型）間で解析領域に変異がなかったことから、
ザゼンソウ属 3 種には明確な遺伝的差違が存在す
る可能性が示唆された。

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